

REFERENCES

- [0048] Chahal P S, Schulze E, Tran R, Montes J, Kamen A A. (2014) Production of adeno-associated virus (AAV) serotypes by transient transfection of HEK293 cell suspension cultures for gene delivery.
- [0049] Chiorini J A, Kim F, Yang L, Kotin R M. (1999) Cloning and characterization of adeno-associated virus type 5.
- [0050] Coleman, J. E., Huentelman, M. J., Kasparov,S., Metcalfe, B. L., Paton, J. F., Katovich, M. J., Semple-Rowland, S. L., and Raizada, M. K. (2003). Efficient large-scale production and concentration of HIV-1-based lentiviral vectors for use in vivo. *Physiol Genomics* 12, 221-228.
- [0051] Cortin V, Thibault J, Jacob D, Gamier (2004) A. High-titer adenovirus vector production in 293S cell perfusion culture. *Biotechnol Prog.* 20(3):858-63.
- [0052] Dormond E, Perrier M, Kamen A. (2009) From the first to the third generation adenoviral vector: what parameters are governing the production yield? *Biotechnol Adv.* 27(2):133-44.
- [0053] Ferreira T B, Ferreira A L, Carrondo M J, Alves P M. (2005) Effect of re-feed strategies and non-ammoniumogenic medium on adenovirus production at high cell densities. *J Biotechnol.* 119(3):272-80.
- [0054] Follenzi, A. and Naldini, L. (2002). Generation of HIV-1 derived lentiviral vectors. *Methods Enzymol.* 346, 454-465.
- [0055] Geraerts, M., Michiels, M., Baekelandt, V., Debyser, Z., and Gijsbers, R. (2005). Upscaling of lentiviral vector production by tangential flow filtration. *J. Gene Med.* 7, 1299-1310.
- [0056] Iyer P, Ostrove J M, Vacante D. (1999) Comparison of manufacturing techniques for adenovirus production. *Cytotechnology.* 30(1-3): 169-72.
- [0057] Karolewski, B. A., Watson, D. J., Parente, M. K., and Wolfe, J. H. (2003). Comparison of transfection conditions for a lentivirus vector produced in large volumes. *Hum. Gene Ther.* 14, 1287-1296.
- [0058] Kamen A, Henry O. (2004) Development and optimization of an adenovirus production process. *J Gene Med.* 6 Suppl 1:S184-S192.
- [0059] Koldej, R., Cmielewski, P., Stocker, A., Parsons, D. W., and Anson, D. S. (2005). Optimisation of a multiparite human immunodeficiency virus based vector system; control of virus infectivity and large-scale production. *J. Gene Med.* 7, 1390-1399.
- [0060] Kuroda, H., Kutner, R. H., Bazan, N. G., and Reiser, J. (2008). Simplified lentivirus vector production in protein-free media using polyethylenimine-mediated transfection. *J. Virol. Methods.*
- [0061] Lenaertz, A., Knowles, S., Drugmand, J C., and Castillo, J. (2013) Viral vector production in the Integrity® iCELLis® single-use fixed-bed bioreactor, from bench-scale to industrial scale. 7 (Suppl 6), 59-60. Jan. 1, 2013. *BMC Proceedings.*
- [0062] Lesch H P, Heikkilä KM, Lipponen E M, Valonen P, Müller A, Räsänen E, Tuunanan T, Hassinen M M, Parker N, Karhinan M, Shaw R, Ylä-Herttuala S. Process Development of Adenoviral Vector Production in Fixed Bed Bioreactor: From Bench to Commercial Scale. *Hum Gene Ther.* 2015 Aug.; 26(8):560-71. doi: 10.1089/hum.2015.081.
- [0063] Liu H, Liu X M, Li S C et al. (2009) A high-yield and scaleable adenovirus vector production process based on high density perfusion culture of HEK 293 cells as suspended aggregates. *J Biosci Bioeng.* 107(5):524-9.
- [0064] Meuwly F, Ruffieux P A, Kadouri A, von SU. (2007) Packed-bed bioreactors for mammalian cell culture: bioprocess and biomedical applications. *Biotechnol Adv.* 25(1):45-56.
- [0065] Naldini, L., Blomer, U., Gage, F. H., Trono, D., and Verma, I. M. (1996a). Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. U. S. A* 93, 11382-11388.
- [0066] Petiot E, Cuperlovic-Culf M, Shen C F, Kamen A. (2015) Influence of HEK293 metabolism on the production of viral vectors and vaccine. *Vaccine.* Nov. 4; 33(44): 5974-81.
- [0067] Rajendran R, Lingala R, Vuppu S K et al. (2014) Assessment of packed bed bioreactor systems in the production of viral vaccines. *AMB Express.* 4:25.
- [0068] Reiser, J. (2000). Production and concentration of pseudotyped HIV-1-based gene transfer vectors. *Gene Ther.* 7, 910-913.
- [0069] Rodrigues A F, Carmo M, Alves P M, Coroadinha A S. (2009) Retroviral vector production under serum deprivation: The role of lipids. *Biotechnol Bioeng.* 104 (6):1171-81.
- [0070] Segura, M. M., Garnier, A., Durocher, Y., Coelho, H., and Kamen, A. (2007). Production of lentiviral vectors by large-scale transient transfection of suspension cultures and affinity chromatography purification. *Biotechnol. Bioeng.* 98, 789-799.
- [0071] Sena-Esteves, M., Tebbets, J. C., Steffens, S., Crombleholme, T., and Flake, A. W. (2004). Optimized large-scale production of high titer lentivirus vector pseudotypes. *J. Virol. Methods* 122, 131-139.
- [0072] Slepushkin, V., Chang, N., Cohen, R., Gan, Y., Jiang, B., Deausen, D., Berlinger, D., Binder, G., Andre, K., Humeau, L., and Dropulic, B. (2003). Large-scale Purification of a Lentiviral Vector by Size Exclusion Chromatography or Mustang Q Ion Exchange Capsule. *BioProcessing Journal* 2, 89-95.
- [0073] Tiscornia, G., Singer, O., and Verma, I. M. (2006). Production and purification of lentiviral vectors. *Nat. Protoc.* 1, 241-245.
- [0074] Wu S C, Huang G Y, Liu J H. (2002) Production of retrovirus and adenovirus vectors for gene therapy: a comparative study using microcarrier and stationary cell culture. *Biotechnol Prog.* 18(3):617-22.
- [0075] Wang, X., Olszewska, M., Qu, J., Wasielewska, T., Bartido, S., Hermetet, G., Sadelain, M., and Riviere, I (2015) Large-scale Clinical-grade Retroviral Vector Production in a Fixed-Bed Bioreactor. 38[3], 127-135. Mar. 4, 2015. *J Immunother.*
We claim:
1. A method for manufacturing a recombinant lentiviral vector, the method comprising:
 - (a) mixing PEI and plasmid coding for a recombinant lentiviral vector to form a transfection solution; and then
 - (b) adding line cells to the transfection solution whereby the plasmid transfests the line cells to make producer cells which produce the recombinant lentiviral vector; and